*Journal of Chromatography*, 482 (1989) 343–350
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 21 546

# INJECTION PEAKS IN ANION CHROMATOGRAPHY

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## SUMMARY

When sample solutions of salts containing eluent ions in the same concentration and pH as the mobile phase are injected into a column, they yield positive injection peaks that are quantitatively related to the sample peak areas. Injection peak areas in anion chromatography are linearly related to the cation concentration of the salts injected. Binary mixtures of salts in the presence of moderate amounts of acid or base can be quantitated by the combination of information from injection and sample peak areas.

## INTRODUCTION

In single-column ion chromatography (SCIC) the first peak is always the injection peak, which is caused by the displacement of eluent ions by the injected sample. It may be either positive or negative, depending on the concentration of the injected sample to which this peak has been shown to be quantitatively related[1]. This has, however, never been fully interpreted, and many chromatograms shown in the literature[2] do not make use of the first few minutes of the chromatographic separation, although the injection peak, which occurs in that period, is potentially a rich source of information.

Several investigators have dealt with the appearance of chromatographic peaks other than sample peaks, namely injection and system peaks. They have pointed out that these are due to the fact that the eluent contains more than one component. Strahanan and Deming[4] explained them as being caused by the change that occurs in the distribution of mobile phase components following sample injection. Levin and Grushka[5] have dealt with system peaks occurring in the chromatographic separation of amino acids when using acetate buffers as eluents. Hummel and Dreyer[6] have applied gel permeation chromatography to the investigation of protein binding to small molecules. They showed that the appearance of an injection and a system peak is the result of injecting a protein sample and a ligand into a column pre-equilibrated with that ligand and in the same concentration.

Two effects contribute to the area of the injection peak, *viz.*, the displacement of

0021-9673/89/$03.50 

eluent ions, caused by the adsorption of the sample ions on the ion-exchange column, and the dilution of the eluent ions, which occurs when the sample is more dilute than the eluent. The first effect tends to increase the area of the injection peak, while the second effect tends to reduce it. Negative injection peaks are observed in many chromatograms cited in the literature[3].

The second effect can be overcome by preparing the sample so that its concentration of eluent ions is the same as that of the eluent itself. The result is a positive injection peak which can be related to the concentration of the sample.

It was the aim of this work to show that the injection peak area of a salt in anion chromatography is proportional to the cationic content of that salt. By combining the information obtained from the injection peak and from the sample peak, mixtures of salts in acidic, alkaline or neutral solutions can be quantitated.

## EXPERIMENTAL

The chromatographic system was built from several components. An LKB2150 high-performance liquid chromatography (HPLC) pump was used to control eluent delivery. A Wescan ICM II ion analyzer with conductivity detection was maintained at a constant temperature. Samples were introduced through a Rheodyne Model 7125 injection valve fitted with a 100-$\mu$l loop. A Shimadzu CR3A integrator was used in conjunction with a Curkin Scientific strip chart recorder. The peak retention times, areas and heights were obtained from the integrator. All separations were effected with a commercial Wescan 269-029 anion-exchange column (25 cm). The flow-rate of the eluent, $1.5 \cdot 10^{-3}$ *M* sodium phthalate (pH 4.3), was maintained at 1.0 ml/min.

Standard solutions were prepared with reagent-grade chemicals and with deionized water (Milli Q reagent grade water system). The concentration of the salt solutions injected ranged from $0.2 \cdot 10^{-3}$ to $1.0 \cdot 10^{-3}$ *M*. All solutions were prepared in $1.5 \cdot 10^{-3}$ *M* sodium phthalate, by the addition of 15.00 ml $1.5 \cdot 10^{-2}$ *M* sodium phthalate before diluting the sample to 100 ml. The injection of each solution was repeated three times, and the average value was used, the relative standard deviation being lower than 2%.

## RESULTS AND DISCUSSION

### *The injection peak*

The size of the injection peak of a salt solution containing the same concentration of the eluent as the mobile phase depends mainly on the cation content of that salt. This is illustrated in Fig. 1, which shows injection peak areas of various sodium salts *vs.* concentration. The linear correlation between the sodium concentration and the peak area is independent of the salt's anion; all points for three different sodium salts fall on one and the same straight line passing through the origin at zero salt concentration. This can be explained as follows: if a sodium salt solution is injected into a chromatographic anion-exchange column, the anion of the salt is retained on the column, displacing eluent ions. In the present case the eluent is sodium biphthalate (buffered at pH 4.30), and biphthalate ions will accordingly be displaced, the injection peak area being proportional to

$$\mathrm{pA} \propto \lambda_{Na^+}[Na^+] + \lambda_{HP^-}[HP^-] + 2\lambda_{P^{2-}}[P^{2-}] \quad (1)$$

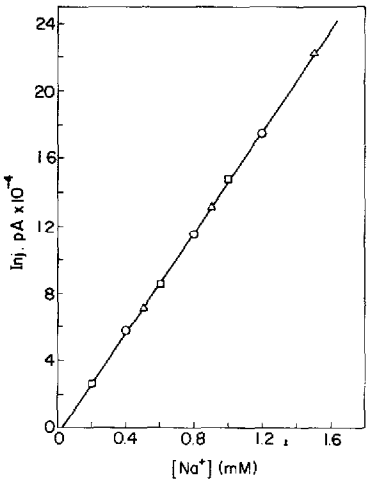



Fig. 1. Injection peak areas of various sodium salts as a function of concentration: □, NaCl; ○, NaOH; △, $NaO_2CCH_3$.

where pA = peak area in arbitrary units; $\lambda_n$ = the equivalent conductivity of the conducting species, $n$, in aqueous solution; and $HP^-$, $P^{2-}$ = biphthalate and phthalate anions.

The area of the injection peak is therefore dependent on the cation content of the sample and on the composition of the eluent before and after injection. It is thus dependent only on the concentration of the anion injected and independent of its type.

*Quantitative relationship between the areas of the injection peak and of the sample peak*

This relationship can best be illustrated by the data in Table I, which shows that there is a certain ratio between the areas of the injection peak and of the sample peak, that ratio being characteristic of the injection of sodium chloride solutions.

The two peaks obtained by injecting only one salt have different meanings.

TABLE I

INJECTION AND SAMPLE PEAK AREAS OF SODIUM CHLORIDE SOLUTIONS

Eluent: $1.5 \cdot 10^{-3}$ *M* NaHP; pH 4.30

| *Concentration of NaCl ($10^3$ M)* | *Injection peak area* | *Peak area of $Cl^-$* | *Ratio between peak areas* |
|---|---|---|---|
| 0.2 | 33 820 (1.0% R.S.D.) | 16 702 (1.2% R.S.D.) | 2.025 |
| 0.4 | 64 544 (0.70%) | 34 731 (0.57%) | 1.86 |
| 0.6 | 94 254 (0.71%) | 53 410 (0.7%) | 1.76 |
| 0.8 | 124 855 (0.41%) | 72 215 (0.12%) | 1.73 |
| 1.0 | 154 971 (0.62%) | 90 625 (0.08%) | 1.71 |

The response of the detector comprising the injection peak area (in arbitrary units) measures the difference between the conductivity of the displaced eluent ions plus that of the sample's cation, and the "background conductivity", *viz.*, that of the eluent, as follows:

$$10^{-3}K\Delta G_{\text{inj.}} = \lambda_{Na^+}[Na^+]_2 + \lambda_{HP^-}[HP^-]_2 + 2\lambda_{P^{2-}}[P^{2-}]_2 - B \quad (2)$$

where the term $B = \lambda_{Na^+}[Na^+]_1 + \lambda_{HP^-}[HP^-]_1 + 2\lambda_{P^{2-}}[P^{2-}]_1$ is the background conductivity, and $\lambda_{Na^+} = 50$, $\lambda_{HP^-} = 38.2$ and $\lambda_{P^{2-}} = 76.4$ cm$^2$ equiv.$^{-1}$ $\Omega^{-1}$, $K$ = conductivity cell constant and $\Delta G_{\text{inj.}}$ = detector response to injection.

The sample peak, for its part, measures the difference between the conductivities of the sample and of the background, respectively. This can be expressed as

$$10^{-3}K\Delta G_s = (\lambda_{Na^+} + \lambda_{Cl^-})I_S C_S - B \quad (3)$$

where $G_s$ = sample peak conductivity, $\lambda_{Cl^-} = 76.3$ cm$^2$ equiv.$^{-1}$ $\Omega^{-1}$, $C_s$ = concentration of the sample and $I_s$ = fractional ionization of the sample (equals unity in the present case).

Dividing eqn. 2 by eqn. 3 gives the ratio of the peak areas as compiled in Table I for the specific case in which the eluent is sodium phthalate at pH 4.30. The injection peak areas of salts other than of sodium can be calculated in the same manner, taking into account the appropriate equivalent conductivity data.

The numerical value of the injection peak conductance, calculated from eqn. 2 for $2 \cdot 10^{-4}$ *M* NaCl, is 0.0182, and for the sample peak is 0.00926. The ratio between the two conductances is 1.965, which is in good agreement with the experimental value given in Table I (2.025). As can be seen from Table I, that ratio decreases somewhat as the concentration of the injected sample is increased. This can be explained by the redistribution of phthalate species with increasing concentration of the injected sample.

*Data for binary salt mixtures*

Fig. 2 shows three chromatograms of the chloride salts of different cations. The chloride concentration is identical for all three salts, and their sample peaks therefore have the same areas; but the injection peaks are different. Potassium chloride, because of its high equivalent conductivity, has the largest peak area. The ratio between the injection peak areas of KCl and NaCl is 121 737/94 254 = 1.292; calculated ratio 1.283. It is thus equal to the ratio of the equivalent conductivities of KHP and NaHP multiplied by the ratio of their concentrations.

The case of $CaCl_2$ is somewhat different. The equivalent conductivity of $Ca^{2+}$ is higher than that of $Na^+$ (59 *vs.* 50 cm$^2$ equiv.$^{-1}$ $\Omega^{-1}$), but the peak area of $Ca^{2+}$ is smaller than that of $Na^+$. The reason is that a divalent ion such as $Ca^{2+}$ will partially interact with $P^{2-}$ to form non-conducting calcium phthalate.

An important fact, however, is that for all three salts the correlation between the cation concentration and the injection peak area is linear, as shown in Fig. 3. Combining the information from the injection peak and sample peak areas enables the composition of binary salt mixtures to be determined, as follows.

Suppose a mixture of KCl and NaCl is injected

$$\text{Injection peak area, Inj.pA} = m[Na^+] + n[K^+] \quad (4)$$

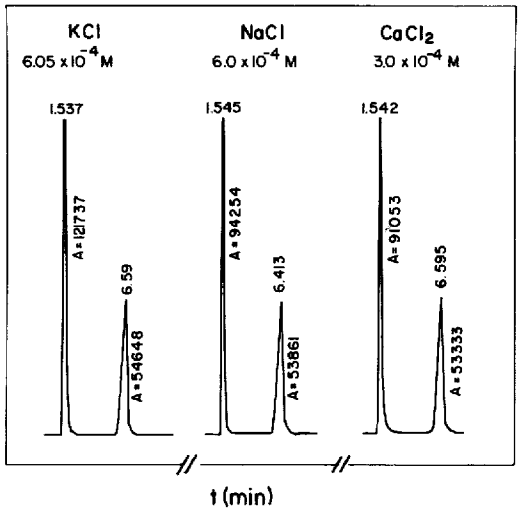



Fig. 2. Injection peaks and sample peaks of three different chloride salts.

where $m$ and $n$ are the respective slopes of the straight lines of the salts in Fig. 3. Then

$$\text{Sample peak area, } pA_{Cl^-} = b([Na^+] + [K^+]) \tag{5}$$

where $b$ is the slope of the calibration line (area *vs.* concentration) of chloride ions (Fig. 5).

There are two unknowns in eqns. 4 and 5, namely $[Na^+]$ and $[K^+]$. They can be accurately determined by measuring the areas of the injection peak and the sample peak and substituting the slopes of the calibration graphs.

Thus, the constituents' concentrations can be derived from eqns. 4 and 5:

$$[K^+] = \frac{\frac{pACl^-}{b} - pAinj}{m - n}$$

$$[Na^+] = \frac{pACl^-}{b} - [K^+]$$

In the case of two salts with two different anions, two sample peaks are available for the determination of the binary mixture. It is therefore quite possible that three salts with different anions can also be determined simultaneously.

*Determination of salt mixtures in the presence of an acid or a base*

Acidic solutions of salt mixtures can be determined quantitatively as described before. When HCl with eluent (NaHP $1.5 \cdot 10^{-3}$ *M*), but no salt, is injected into the column, the injection peak areas are very small, as is seen in Fig. 4. This is because the

TABLE II

QUANTITATION OF A SALT MIXTURE IN THE PRESENCE OF AN ACID OR A BASE

| *Composition of injected sample*[a] | *Inj.pA of mixture* | *Inj.pA of $Na^+$ from Fig. 5* | *Inj.pA $K^+$ from Fig. 5* | *Sum* | *Rel. error (%)* | *Sample peak $Cl^-$* | *Sample peak $Cl^-$ from Fig. 5* | *Rel. error (%)* | *Sample peak $NO_3^-$* | *Sample peak from Fig. 5* | *Rel. error (%)* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| $NaCl + KNO_3$ | 207 485 (0.46% R.S.D.) | 89 275 (0.3%) | 114 737 (0.03%) | 204 010 | 1.7 | 54 468 (1.8%) | 54 307 (0.51%) | 0.296 | 34 338 (1.6%) | 34 266 (0.25%) | 0.21 |
| $NaCl + KNO_3 + NaOH$ | 301 766 (0.2%) | 89 275 × 2 | 114 737 | 293 290 | 2.89 | 53 910 (0.5%) | 54 307 | 0.74 | 35 282 (1.1%) | 34 266 | 2.96 |
| $NaCl + KNO_3 + HCl$ | 208 366 (0.2%) | 89 275 | 114 737 | 204 010 | 2.13 | 111 080 (0.2%) | 54 307 × 2 | 2.27 | 34 471 (1.3%) | 34 266 | 0.59 |

[a] The concentration of each salt, acid or base was $6 \cdot 10^{-4}$ *M*.

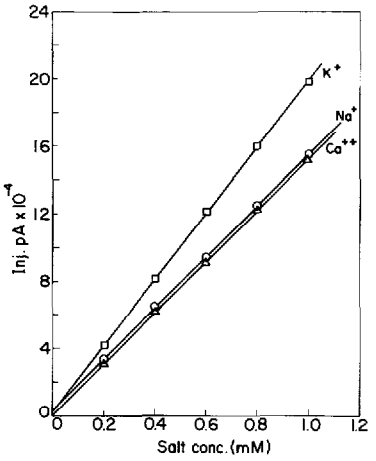
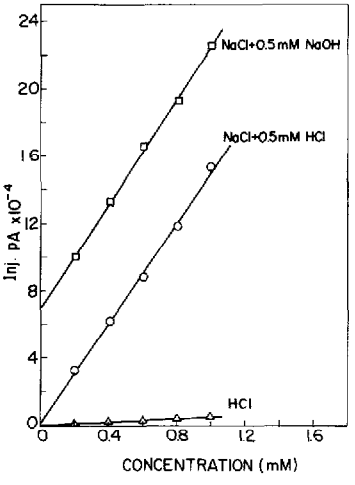



Fig. 3. Injection peaks of salts of $K^+$, $Na^+$ and $Ca^{2+}$ as a function of concentration.

added $H^+$ converts some $HP^-$ into $H_2P$, and the overall effect is therefore small. If salts are injected in the presence of HCl, that effect will be even smaller, because much more $HP^-$ is displaced, and only a small part of it converted into $H_2P$, so that the area of the injection peak of HCl in the presence of salts is negligible. As is also seen from Fig. 4, the straight line plot of the injection peak areas of NaCl + HCl intersects with the origin and is identical with the line for NaCl alone (Fig. 1).

In alkaline solution the addition of $OH^-$ does not appreciably affect the injection peak, because some $HP^-$ is converted into $P^{2-}$, but the sodium ion increases the injection peak area. This is also illustrated in Fig. 4, where a mixture of NaOH and



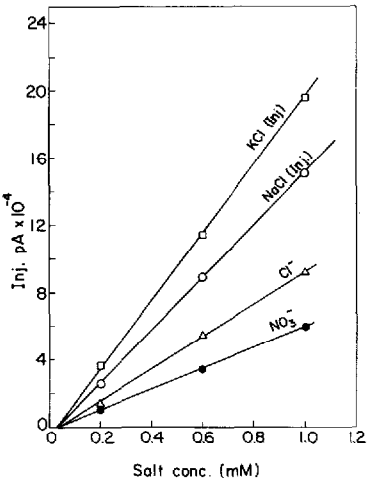




Fig. 4. Injection peak calibration graphs for NaCl in the presence of HCl and NaOH.

Fig. 5. Calibration graphs of injection peaks and sample peaks needed for the quantitation of a mixture of NaCl and $KNO_3$.

NaCl yields a straight line parallel to that for NaCl + HCl; but its intercept is much higher, because the added sodium ions contribute to the injection peak area. There is, of course, a limit to the acid or base content of a sample that can be tolerated.

Experimental results of the quantitation of a solution containing known concentrations of NaCl and $KNO_3$ are shown in Table II. Each determination was made three times, and the data for the chromatographic peaks obtained were compared with those of the calibration lines of Fig. 5. From Table II, there is good correspondence between the experimental data for the mixture and the data obtained by injecting each constituent separately. The relative error of the determinations does not exceed 3%, and is is interesting that the relative standard deviations of the injection peaks are much lower than those of the sample peaks.

## CONCLUSIONS

In single-column ion chromatography the commonly used eluents are salts of weak organic acids, which provide the background conductivity of the eluent. When a salt solution is injected into a chromatographic anion-exchange column, anions of the eluent are displaced from the column, and the constituents of the eluent are redistributed. This change in the momentary composition of the eluent, together with the injected cation (of the sample), contributes to the conductivity of the injection peak.

By taking advantage of quantitative information on cations derived from the injection peak, and for anions from the sample peaks, salt mixtures may be quantitated.

## ACKNOWLEDGEMENTS

This work was supported in part from a grant from the Rohm and Haas Co. Work was performed in the Ames Laboratory, Iowa State University. Ames Laboratory is operated for the U.S. Department of Energy under Contract No. W-7405-ENG-82.

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